

WHAT IS CLAIMED IS:

1. A method for detecting a mutation and/or a SNP in a double-stranded test DNA molecule, comprising:

- (a) providing a single stranded DNA probe which is optionally detectably labeled, which probe has (i) a known nucleotide sequence or (ii) a sequence complementary to the sequence of at least a part of the test DNA;
- (b) contacting the probe with a RecA protein which is optionally detectably labeled, to form a RecA filament;
- (c) contacting the RecA filament with the test DNA, thereby forming a three stranded DNA D-loop structure in the test DNA, which D-loop structure comprises the probe and the two strands of the test DNA;
- (d) contacting the DNA D-loop structure with a MutS protein which is optionally detectably labeled, wherein the MutS binds to one or more base pair mismatches or unpaired bases present in the duplex portion of D-loop structure;
- (e) detecting the presence of MutS bound to the DNA D loop structure,
wherein the presence of the bound MutS is indicative of the presence of the mutation or the SNP in the test DNA.

2. A method for detecting a mutation and/or a SNP in a double-stranded test DNA molecule, comprising:

- (a) providing a probe comprising two complementary single stranded oligonucleotides which are optionally detectably labeled, which probe has a known nucleotide sequence or a sequence complementary to the sequence of at least a part of the test DNA;
- (b) contacting each of the oligonucleotides in single stranded form with a RecA protein, which is optionally detectably labeled, to form RecA filaments,
- (c) contacting the RecA filaments with the test DNA, thereby forming a four stranded DNA structure in the test DNA, which structure comprises the probe and the two strands of the test DNA and wherein the probe strands are annealed with test DNA strands;

- (d) contacting said DNA structure with a MutS protein which is optionally detectably labeled, wherein the MutS binds to one or more base pair mismatches or unpaired bases present in the four stranded DNA structure;
- (e) detecting the presence of the MutS bound to the DNA structure

wherein the presence of the bound MutS is indicative of the presence of the mutation or the SNP in the test DNA.

3. A method for detecting a mutation and/or a SNP in a double-stranded test DNA molecule, comprising:

- (a) providing a single stranded DNA probe which is optionally detectably labeled, which probe has (i) a known nucleotide sequence or (ii) a sequence complementary to the sequence of at least a part of the test DNA molecule;
- (b) contacting the probe with a RecA protein which is optionally detectably labeled, to form a RecA filament,
- (c) contacting the RecA filament with the test DNA, thereby forming a three stranded DNA D-loop structure in the test DNA, which structure comprises the probe and two strands of the test DNA;
- (d) contacting the DNA D-loop structure with immobilized MutS which binds to one or more base pair mismatches or unpaired bases present in the duplex portion of the D-loop structure;
- (e) detecting the presence immobilized probe DNA or RecA bound to the MutS,

wherein the presence of the bound probe DNA or RecA is indicative of the presence of the mutation or the SNP in the test DNA.

4. A method for detecting a mutation and/or a SNP in a double-stranded test DNA molecule, comprising:

- (a) providing a probe comprising two complementary single stranded oligonucleotides which are optionally detectably labeled, which probe has a known nucleotide sequence or a sequence complementary to the sequence of at least a part of the test DNA;

(b) contacting each of the probe oligonucleotides in single stranded form with a RecA protein, which is optionally detectably labeled, to form RecA filaments,

(c) contacting the RecA filaments with the test DNA, thereby forming a four stranded DNA structure in the test DNA, which structure comprises the two strands of the test DNA to each of which is annealed a probe oligonucleotide strand; and

(d) contacting said DNA structure with immobilized MutS which binds to one or more base pair mismatches or unpaired bases present in said four stranded DNA structure, thereby detecting the mutation and/or SNP.

5. The method of any of claims 1-4, wherein the mutation being detected is a single nucleotide substitution or the addition or deletion of 1-4 nucleotides.

6. The method of any of claims 1-4, wherein the test DNA molecule is selected from the group consisting of prokaryotic genomic DNA, eukaryotic genomic DNA, cDNA, viral DNA, plasmid DNA, and a DNA fragment amplified by PCR or by another amplification method.

7. The method of any of claims 1 - 4, wherein the probe is selected from the group consisting of:

- (a) a synthetic oligonucleotide;
- (b) a recombinant oligonucleotide;
- (c) an oligonucleotide obtained by denaturing, and, optionally cleaving, a double stranded DNA molecule.

8. The method of claim 7, wherein the oligonucleotide has a length of about 20 to about 60 nucleotides.

9. The method of any of claims 1 – 4, wherein:

- (i) the probe and the MutS are labeled;
- (ii) the label is a fluorophore, a chromophore, a radionuclide, biotin or digoxigenin; and

(iii) association of the probe label with the MutS label is indicative of the presence of the mutation or the SNP in the test DNA.

10. The method of any of claims 1 - 4, wherein the RecA protein is from *E. coli*.

11. The method of any of claims 1 - 4, wherein

- (i) the RecA and MutS are labeled;
- (ii) the label is a fluorophore, a chromophore, a radionuclide, biotin or digoxigenin; and
- (iii) association of the RecA label with the MutS label is indicative of the presence of the mutation or the SNP in the test DNA.

12. The method of claim 1 or 2 wherein the MutS is immobilized to a solid support.

13. The method of claim 1 or 2 wherein the detectable MutS label is a fluorophore, a chromophore, a radionuclide, biotin, digoxigenin, a detectably labeled bead, a detectable labeled anti-MutS antibody, or a combination of an unlabeled anti-MutS antibody and a detectably labeled secondary antibody specific for the anti-MutS antibody.

14. The method of claim 1 or 2 wherein the RecA protein is labeled and the detection is of the MutS label associated with the RecA label present in the DNA D loop structures.

15. The method of any of claims 1 - 4, wherein the detectable RecA label is in the form of a detectably labeled primary anti-RecA antibody, or a combination of an unlabeled anti-RecA antibody and a detectably labeled antibody specific for the anti-RecA antibody.

16. The method of any of claims 1-4, wherein one or more of the detectably labeled probe, the detectably labeled RecA and/or the detectably labeled MutS is labeled with a fluorophore.

17. The method of any of claims 1-4, wherein the detecting is by flow cytometry.

18. The method of claim 16 wherein the detecting is by flow cytometry.

19. The method of claim 1 or 3 wherein the DNA D loop structure is stabilized by the addition, before step (d), of SSB protein which is optionally detectably labeled.

20. The method of claim 19, wherein,

- (i) the SSB protein is labeled with a detectable label;
- (ii) the label is a fluorophore, a chromophore, a radionuclide, biotin, digoxigenin, a labeled anti-SSB antibody, or a combination of an unlabeled anti-SSB antibody and a labeled secondary antibody specific for the anti-SSB antibody; and
- (iii) association of the SSB label with the MutS label is indicative of the presence of the mutation or the SNP in the test DNA.

21. The method of claim 1 or 3 wherein the detecting is by flow cytometry which detects the coincidence of two, three or four labels which are bound to:

- (a) MutS and the probe;
- (b) MutS and RecA;
- (c) MutS, RecA and the probe;
- (d) MutS and SSB;
- (e) MutS, SSB and the probe; or
- (f) MutS, SSB, the probe and RecA.

22. The method of any of claims 1-4 wherein the probe is labeled by polymerase extension using labeled deoxynucleotide triphosphates or nucleotide terminators.

23. The method of claims 1 or 2, wherein the test DNA is immobilized to a solid support.

24. The method of claim 1 or 2, wherein the probe is bonded to an adduct that allows immobilization of the probe following formation of said D-loop structure.

25. The method of claim 24, wherein the adduct is an oligonucleotide.

26. The method of claim 24, wherein the adduct is biotin or digoxigenin.

27. A kit useful for detecting a one or more mutations or polymorphisms in a DNA sample, the kit being adapted to receive therein one or more containers, the kit comprising:

- (a) a first container containing a RecA protein which is optionally detectably labeled;
- (b) a second container containing MutS protein which is optionally detectably labeled; and
- (c) a third container or plurality of containers containing buffers and reagent or reagents capable of detecting bound MutS.

28. A kit useful for detecting a specific mutation or polymorphism or a specific group of mutations or polymorphisms in a DNA sample or for examining a specific region or regions of DNA for any mutations or polymorphisms, the kit being adapted to receive therein one or more containers, the kit comprising:

- (a) a first container containing RecA protein which is optionally detectably labeled;
- (b) a second container containing MutS protein which is optionally detectably labeled;
- (c) a third container or plurality of containers containing a specific oligonucleotide probe or probes, which probes are selected to be complementary to specific sequences in specific regions in the DNA of the sample and which form mismatch-containing or unpaired base-containing heteroduplexes with a mutated or polymorphic sequence or sequences in the specific DNA regions, which probe or probes is or are optionally detectably labeled; and
- (d) A fourth container or plurality of containers containing buffers and reagents capable of detecting MutS when it is bound to specific heteroduplexes formed between the probes and the sample DNA.